"Phytochemical Investigations and Screening for Analgesic, Anti-pyretic and anti-inflammatory Activity of Stem and Fruit of *Parkinsonia aculeata linn.* and Leave of *Rotula aquatica lour* ".

A

Synopsis submitted to

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for the fulfillment of

Doctor of Philosophy

In

Pharmacy

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Jan. 2020

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1. **INTRODUCTION**:

1.1 **Introduction to Herbal Medicine**

World is endowed with a rich wealth of medicinal plants. Herbs have always been the principle form of medicine in India and presently they are becoming popular throughout the developed world, as the people strive to stay healthy in the face of chronic stress and pollution and to treat illness with medicines that work in concern with the body's own defenses. Herbal medicines generally have fewer side effects than synthetic compounds, and their effectiveness can be improved by modern pharmacological[1].

Importance of Plant Based Drugs:

An estimated 80% of the population in developing countries still depends on the traditional medicines for their primary health care needs and higher plants are the main source of their medicinal preparations. As per the data from global survey, it was found that at least 130 plants were used in modern medicine.

Reasons for Faster Expansion of Plant Drug Market:

- ✓ Realization of harmful side effects from synthetic drugs.
- ✓ Inefficiency of allopathic drugs against certain diseases.
- ✓ Plant drugs are comparatively less expensive.
- ✓ Western population is demanding for herbal formulations.

➢ Pharmaceutical industries are giving importance for plant drugs in their search for new molecules.

Undoubtedly plants have provided useful drugs to mankind for their health care and other needs. The efforts to combat the disease for which there is no satisfactory solution as yet, should be continued relentlessly. Hopefully, plant kingdom will help us in the discovery of new drugs useful for the alleviation of human illness.[2]

1.2 Introduction of Analgesics:

Pain - Generic History

Pain is a disabling accompaniment of many medical conditions and pain control is one of the most important therapeutic priorities.

Pain is a subjective experience, hard to define exactly, even though we all know what we mean by it. Typically, it is a direct response to an untoward event associated with tissue damage such as injury, Inflammation or cancer! Pain conditions of the latter kind, not directly linked to tissue injury, are very common and a major cause of disability and distress. Therefore it is useful to distinguish two components, either or both of which may be involved in pathological pain states.[3]

1.3 Introduction to Anti-pyretic activity:

Introduction of fever:

Fever may be due to infection or one of the sequels of tissue damage, inflammation, graft rejection, or other disease states. Antipyretic are agents, which reduce the elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained.[4]

In fever this set point elevates and a drug like paracetamol does not influence body temperature when it is elevated by the factors such as exercise or increase in ambient temperature. Yeast induced fever is called pathogenic fever. Its etiology includes production of prostaglandins, which set the thermoregulatory center at a lower temperature. Elevated body temperature and pain are two major signs of the body against inflammation[5].

1.4 Anti- Inflammatory Activity

Introduction to Inflammation

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. It is a body's defense reaction in order to eliminate or limit the spread of injurious agent as well as to remove the consequent necrosed cells and tissues.

Inflammation is a defensive reaction of the local microcirculation to tissue injury arising from cell damages due to mechanical trauma, chemical, physical and thermal injury, antigen antibody reactions and infections. The signs and symptoms of

inflammation include redness, swelling, heat, pain and loss of function of the affected area. Pain is an unpleasant sensation that is a consequence of complex neurochemical processes in the central and peripheral nervous systems. Non-steroidal anti-inflammatory drugs (NSAIDS) and opioids are used in management of mild to moderate and severe pains respectively. It is therefore, inevitable to search for new, less toxic and more effective anti-inflammatory and analgesic agents.

The agents causing inflammation may be as under:

1. Physical agents like heat, cold, radiation, mechanical trauma.
2. Chemical agents like organic and inorganic poisons.
3. Infective agents like bacteria, viruses, and their toxins.
4. Immunological agents like cell-mediated and antigen mediated antibody reactions.

Analgesic - Antipyretic – Anti-inflammatory drugs (NSAIDS):

Effects of NSAIDS:

A wide variety of molecules which are structurally fairly simple have powerful multiple effects, making them useful for alleviation of pain, reduction of fever, and suppression of inflammation; they are most commonly known as NSAIDs (non-steroidal anti-inflammatory drugs). Historically the most important is salicylic acid, a compound common in plants (notably willow bark) and used for centuries as a traditional medicine; it is still the cheapest and most readily available, primarily as the acetate ester known as aspirin. Among the side effects of NSAIDs are severe gastrointestinal disturbance and inhibition of the platelet aggregation necessary for blood clotting; the inhibition of clotting has a positive side - reduction in heart attack frequency for people on aspirin who are otherwise at risk[6].

2. Aim and Objectives:

2.1 Aim of the Study

Aim:

Phytochemical Investigations and Screening for Analgesic, Anti-pyretic and anti-inflammatory Activity of Stem and Fruit of *Parkinsonia aculeata linn.* and Leave of *Rotula aquatica lour.*

2.1.1 To carry out Pharmacognostic evaluation of fruit and stem *P. aculeata* and leave of *R. aquatica.*

2.1.2 To carry out phytochemical evaluation of fruit and stem *P. aculeata* and leave of *R. aquatica.*

2.1.3 To carry out anti-inflammatory, analgesic, antipyretic and antioxidant activity of

Different extracts of fruit and stem *P. aculeata* and leave of *R. aquatica.*

2.2 Objectives of the Study:

Today, there are very old traditions and records of popular healing methods that have maintained their importance despite new developments and progress in the field of Chemistry, Pharmacy and Medicine. Indeed, interest in herbal drugs is increasing. The role of Natural Products in the development of drugs used in modern medicine is unsurpassed even when synthetic chemistry has been developed beyond expectations.

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. It is the body's defense reaction in order to eliminate or limit the spread of injurious agent as well as to remove the consequent necrosis cells and tissues.

Fever may be due to infection or one of the sequels of tissue damage, inflammation, graft rejection, or other disease states. Antipyretic are agents, which reduce the elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained.[7]

All parts of *P. aculeata Linn* are reported to be used as antipyretic; fruit and stem are considered as diaphoretic and abortifacient and *P. aculeata* contain various flavonoids, flavonols and flavones according to the literature (orientin, iso-orientin, vitexin, iso-vitexin, lucenin-II, vicenin-II, diosmetin 6-C-B-glucoside, apigenin, luteolin, 7-glycosyl kaempferol, chrysoeriol, epi-orientin, Parkinsonin-A, Parkinsonin-B and Parkintin and reported, anti-inflammatory, analgesic activity on Fruit And Stem parts of *P. aculeata* but no scientific reports are available for the same, hence to give a scientific back ground to the above traditional claim this work has been taken up. [8-9]

Survey of the genus Parkinsonia revealed the presence of variable classes of compounds such as flavones, flavanones, flavone Cglycoside, alkaloids, sterols, tannins, terpenoids, glycerols, carotenoids, saponins, carbohydrates, free amino and fatty acids. P. aculeata L. has several reported health merits e.g., hypoglycemic (aqueous fraction of aerial part and bark), hepatoprotective (leaves extract), antispermatogenic (ethanolic extract of stem bark), antimalarial (flowers and leaves), amoebicidal,smooth muscle stimulant, analgesic, anti-inflammatory, antipyretic (total alcoholic and aqueous extract of leaves) activities.

R. aquatica reported that the plant is a mandatory component of many ayurvedic drug preparations and is an important traditional medicine for kidney and bladder stones.

Pharmacological activity may be due to the presence of alkaloids, phenolic compounds, flavonoids, and other nutrients like as amino acid and proteins. The aqueous extracts of *Rotula aquatica* was reported to contain polyphenols. The Whole Plant tuber is used in fever and reported anti-inflammatory activity[10] on this plant but no scientific reports are available for the same, hence to give a scientific back ground to the above traditional claim and papers so this work has been taken up.

Phytochemical and Pharmacological investigations have added a great deal of status to the use of medicinal plants by revealing the presence of active principles and their actions on human and animal systems. The objective of our study is to evaluate the Pharmacognostic and Phytochemical character and analgesic, anti-pyretic, anti-inflammatory activity of the alcoholic and aqueous extracts of Fruit and Stem of *P. aculeata* and Leave of *R. aquatica.*

3. Introduction to Plants:

3.1 *Parkinsonia aculeata.* Linn[11].

Part selected for proposed

study: Fruit and Stem

Photo No .3. Stem of *P. aculeate*

Division: Magnoliophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Subfamily: Caesalpinioideae

Genus: *Parkinsonia*

Species: *P. aculeate*

Photo No.4. Fruit of *P. aculeata* in natural habitat

Vernacular Names:

Kannada : Parangi jali,

Hindi : Vilayati kikar,

vilayati babul

Telugu : Sima tumma

Marathi : Adanti

Nature: A large spinous shrub or a small tree, native of tropical America, found almost throughout the drier parts of India. It may have single or multiple stems and many branches. Younger **stems** have a pair of sharp spines (3-20 mm long) below each leaf and these remain on older **stems** after the leaves. flowers in ax axillary racemes, yellow, fragrant; pods slender, moniliform, upto 10 cm. Long; Bark green or brown, thin, smooth; leaves bipinnate, ending in a stout spine: 15-30 cm long, pinnules ovate or oblanceolate, minute; seeds usually 4-7, oblong, smooth dark brown.

Ethnobotanical used[12-13]: All parts of plant are reported to be used as antipyretic; Stem are considered as diaphoretic and abortifacient.

Scientific reports:

Sahar Abdelaziz et al[14] reported that Phytochemical profile, antioxidant and cytotoxic potential of Parkinsonia aculeata L. growing in Saudi Arabia Parkinsonia aculeata L. growing in Saudi Arabia was investigated for its phytochemical profile, antioxidant,and cytotoxic properties. UPLC-ESI-MS/MS was employed as a powerful

technique for the characterization of secondary metabolites from a hydroalcoholic extract, dichloromethane, and ethyl acetate
fractions of P. aculeata L. aerial parts. Sixty-nine compounds (flavonoids, anthocyanins, phenolics andfatty acids) were detected and characterized; flavonoids were the abundant components in the analyzedsamples.

Sonia Sharma et al[15] reported that Preliminary Phytochemical Screening and In Vitro Antioxidant Activities of Parkinsonia aculeata Linn Butanol and hexane leaves extracts of Parkinsonia aculeata L. (Fabaceae) were assessed for its antioxidant potential by in vitro methods. Phytochemical analysis and antioxidant activity of plant extracts were studied using different in vitro assays. UPLC analysis of extracts was carried out for the identification of chemical constituents.

Fatemeh Mirzaee et al.[16] reported that Parkinsonia aculeata and Prosopiskoelziana are two spinous ornamental plant from Leguminosae family. Besides their traditional uses, many pharmacological activities have been reported from family members, although little studies have been done about their antimicrobial properties.Natural products especially plant sources are under great considerations of public and medical experts.

B.Divya et al.[17] reported that since the Ancient period plant have been utilized by human being have been as medicinal agents on the basis of ethno medical background later on these speculations ware transformed into a specific basic as a single drug agents.

Ghalib l. Hundekari et al.[18] reported that The anti-inflammatory effect of the pet ether and aqueous leaf extract of Parkinsonia acculeata was evaluated using the carrageenam and formalin inducedrat paw edema.

Green[19] reported that the composition of the pods of *P. aculeata.* The 73% of pods consist of hemicellulose, cellulose and lignins. The composition of whole bean is similar to that of alfalfa hay or mesquite beans. The chief proteins of the seed are albumin and glutelin.

Tookey[20] et al. reported that the seeds of 175 species in 26 plant families for water-soluble mucilage content. Those containing over 15% of mucilage were all from legumes. Out 175 species, seeds of *Parkinsonia aculeata* reported to contain 28% (Percentage of seed) of mucilage, which was highest.

Trivedi & Schemes[21] presented that the Pharmacognostical data on *Parkinsonia aculeata* as well as its use as an antirabies agent based on folklore with clinical data involving 3 patients (ages 14, 28, and 40 yr). The drug was given orally with water in the form of expressed juice or as a fine powder.

Ali et al[22] reported that a new flavanone with epoxy-isopentyl moiety named parkintin has been isolated from the methanol soluble part of Parkinsonia aculeata Linn. belonging to the family Caesalpiniaceae. The structure of parkintin has been established with the aid of spectroscopic techniques including COSY and HMBC experiments.

Singh et al[23] reported that the present study evaluation the qualitative analysis of phytochemical and antibacterial activity of chloroform leaves extract of Parkinsonia aculeata L family (Leguminoseae) leaves against some bacteria causing Urinary tract infection in human.The preliminary phytochemical screening show the presence of flavonoids, alkaloids, C-glycoside and Saponins.The crude extract show maximum anti bacterial activity against E- Coli with zone inhibition 23±0.2 at concentration of 500 mg/ml.

Besson et al[24] reported that isolated the C-glycosylflavones from *Parkinsonia aculeata* leaves. The isolated compounds were identified as orientin, isoorientin, vitexin and isovitexin. They also reported that Orientin was reported to be present in the leavorotatory form.

3.2. *Rotula aquatica* lour[25].

Synonym: Carmona viminea (Wallich), Ehretia viminea Wallich, Rhabdia viminea (Wallich) D.

Family: Boraginaceae.

Part Used: leave

Fig No. 5 Fresh leaves and flower of *Rotula aquatica*

Division: Angiosperms

Class: Asterids

Order: (unplaced)

Family: Boraginaceae

Subfamily: Ehretioideae

Genus: *Rotula*

Species: ***R. aquatica***

Vernacular Names:

Kannada: Paashaanabheda,

Hindi: Pashanabhed

Telugu: Pashanabhedi

Marathi: Machim

Tamil : Ceppunerinji

Malayalam: Kalluranci

Sanskrit: Ashmahabhedah

Nature[26]: A small much branched shrub, 60-180 cm high with numerous short lateral arrested branchlets off ten rooting; leaves simple, nearly sessile, spathulate, rouded at the apex, more or less hairy, crowded on the branches; flowers pink or reddish, shortly pedicellate, single or 2-3 together on short lateral branches, stamens exerted beyond the corolla tube; fruits subglobose, orange red drupes, tipped with the remains of the style, Stems gray or black-gray, glabrous. Petiole 0.5-4 mm; leaf blade oblong or oblanceolate, 0.5-2.5 cm × 2-10 mm, ± leathery, strigose, base rounded, cuneate to broadly cuneate, margin entire, apex obtuse, mucronate. Corolla 6-7 × 6-7 mm. Fruit yellow- to brownish red, ca. 4 mm in diam., nearly dry.

Distribution[27]: ***Rotula aquatica*** is a species of aromatic flowering shrub in the borage family, Boraginaceae. It is a rare rheophyte native to India, where it is a member of the lotic ecosystem of streams.

Ethnobotanical uses:

Sivarajan and Indira Balachandran[28] (Ayurvedic Drugs and Their Plant Sources.*)* reported that the plant is a mandatory component of many ayurvedic drug preparations and is an important traditional medicine for kidney and bladder stones. The root tuber is astringent, bitter, diuretic and also useful in treating coughs, heart diseases, dysuria, blood disorders, fever, poisonings, ulcers and uterine diseases.

Website[29], the ethnobotanical uses of *Rotula aquatica* were reported. Those are as follows.

- **Indian-herbs-exporters[30]** reported that the leaves are bitter, astringent. cooling, diuretic and laxative and are useful in hemorrhoids, renal and vesicle calculi, diabetes and venereal diseases.

Scientific reports:

A.Vysakh et al[31] reported that traditional and Therapeutic Importance of Rotula aquatic Lour.: An Overview. *Rotula aquatica* Lour. is a rare rheophyte belonging to the family Boraginaceae. The plant is a member of lotic ecosystem of streams in India.

Bhanu Priya et al[32] reported that This research aims to detect the *in-vitro* antioxidant activity of the methanolic and aqueous stem extract of *Rotula aquatica lour* and to estimate its phenolic and flavonoid content. DPPH (2, 2-diphenyl-1-picryl hydrazyl), superoxide radical scavenging capacity and reducing power assay (RPA) were carried out to evaluate *in vitro* antioxidant activity. Phytochemical screening was carried out according to standard procedures followed by their TLC profiling was carried out.

Prashanthi P et al[33] reported that Urinary kidney stone formation is multifaceted complex process involving accumulation and aggregation of oxalates and phosphates of calcium, magnesium, ammonium and uric acid. Traditional systems of healing like Ayurveda and Unani have played a praiseworthy role from time immemorial in treatment of kidney stones. One such a plant is *Rotula aquatica* Lour. belonging to Borage family.

Vijayakumari et al[34] reported that Molecular docking is a bioinformatics tool used to study and analyse ligand receptor interactions. This helps in identifying the receptors (molecular targets) for differentligands. Using these technologies, compound isolation and drug discovery from herbals is achieved. Herbs are widely used in treatment of various ailments from timeimmemorial. Phytochemists and drug developers are now interestingly working indeveloping new molecules that can act effectively than conventional drugs.

Bency Baby T et al[35] reported that *Rotula aquatic* Lour is an important medicinal herb in Ayurvedic system of medicine. Theplant occurs as a small branched shrub that grows characteristically among rocks and trailingover pebbles in stream beds, belonging to the family Boraginaceae and widely distributed throughout India. It is commonly called as Pasanabheda in Ayurveda.

3.3 Review of literature for Analgesic-Antipyretic-Anti-inflammatory activity:

Esther et al[36] reported that despite the progress made in medical research in the past decades, the treatment of many diseases including inflammatory diseases is still problematic. Conventional drugs used to ameliorate these conditions are either too expensive or toxic, there is therefore an urgent need to search for newer, cheaper and safer medications. *Strophanthus sarmentosus* (SMS) is an indigenous plant used in traditional medicine in West Africa for the treatment of inflammatory diseases among other uses. The present study was designed to explore its therapeutic benefits in inflammation, nociception and pyrexia. Analgesic effect of the methanolic extract of dried SMS root (50, 100 and 200 mg kg-1) was evaluated in mice using acetic acid-induced writhing and hot plate models, while the efficacy of the herbal drug was compared with 150 mg kg-1 acetylsalicylic acid, 0.5 mg kg-1 piroxicam and 5 mg kg-1 morphine respectively.

Venkataraman et al[37] reported that the pharmacological and chemical constituents of plants from the plant *Pergularia daemia* Forsk which is widely used in folk medicine. In the present study, the analgesic, antipyretic, anti-inflammatory of Petroleum ether and Chloroform extract of plant of *Pergularia daemia* Forsk were studied .The analgesic activity was found out by eddy's hot plate method by using standard Diclofenac sodium . The antipyretic activity was found out by yeast induced pyrexia method by using standard Paracetamol. The anti-inflammatory activity was found out by Carragenan induced paw edema method by using standard Diclofenac sodium. Preliminary phyto chemical screening showed the presence of glycosides, alkaloids, phytosterols, saponins, fixed oils and fats, flavonoids and coumarins are present in extracts. In carrageenan induced paw odema method the chloroform extract of *pergularia daemia* showed more significant inhibition than petroleum ether extract. The results were found to be highly significant ($p<0.01$) in comparison to the control. In eddys hot plate method the chloroform extract of *pergularia daemia* exhibited significant analgesic activity than petroleum extract. In antipyretic activity the chloroform and pet ether both extracts having significant activity.

Jayabharathi et al[38] reported that **t**he ethanolic extract of the flowers of Moringa concanensis (family:Moringaceae) was tested for phytochemicals, anti-inflammatory,

analgesic and antipyretic activity. Qualitative phytochemical analysis of Moringa concanensis flower extract was carried out with a view of developing leads for new therapeutic products. The results indicate that Moringa concanensis is rich in phytoconstituents and the studies also indicate that the ethanolic extract showed significant antiinflammatory, analgesic and antipyretic activity when compared with standard drug.

Fadeyi et al[39] reported that four-Substituted derivatives of salicylic and anthranilic acids: 2-hydroxy-5-azidosulfonylbenzoic acid (HASBA, 1), 2-acetyloxy-5-azidosulfonylbenzoic acid (AASBA, 2), 2-acetamido-5- azidosulfonylbenzoic acid (AMASBA, 3) and 2-acetamido-5-sulfonamidobenzoic acid (AMSABA, 4) were synthesized and evaluated for their analgesic, antipyretic, anti-inflammatory and cytotoxic activities. HASBA, AASBA and AMASBA showed higher analgesic activity than aspirin (ASA) at 100 mg/kg dose, while AMSABA showed the least analgesic property. AMASBA exhibited higher antipyretic activity than paracetamol (PCM), while HASBA, AASBA and AMSABA also showed antipyretic effects which were of equal potency to that of PCM. The order of anti-inflammatory effects of the four compounds is: AASBA > AMASBA > HASBA > AMSABA. The effects of the substituents on the biological activities of the synthesized compounds are discussed.

Clement et al[40] reported that the analgesic activities of the aqueous extract of *Carpolobia lutea* was evaluated in mice and rats using the mouse writhing, tail flick and formalin induced pain tests. Analgesic studies were performed using three models; mouse writhing assay, formalin test and tail flick assay. The extract (1500 to 2500 mg/kg) and acetylsalicylic acid (100 mg/kg) produced a significant ($P<0.05$) inhibition of the second phase in the formalin pain model, while the antinoceptive effect was not produced in the first phase. The extract also showed a dose dependent inhibition of acetic acid induced abdominal writhings. The tail flick latency was not enhanced by the extract. Oral administration of the extract up to 2500 mg/kg did not produce any toxic effects in the acute toxicity studies in mice. The LD50 of the extract when administered orally was 3338.83 mg/kg. The data obtained shows that *C. lutea* posseses analgesic activity that is peripherally mediated.

3.4 Review of literature for Analgesic activity:

Rajesh et al[41] reported that the methanolic extract of the leaves of Hedera helix L. (Araliaceae) which contains mainly saponins and carbohydrate showed significant analgesic and anti-inflammatory activities ($P<0.05$) in the tail flick/ hot-plate test and egg albumen-induced rat paw oedema tests that were comparable to the test drugs (morphine 20mg/kg and indomethacin 50mg/kg respectively). These findings suggest that the methanol leaf extract of Hedera helix L. possess analgesic (which might have been peripherally mediated) and anti-inflammatory activities; and may lend credence to the ethnomedical claim of the use of the plant for the management of pain and inflammatory conditions. al of pharmacy

Lokesh et al[42] reported that *Pergularia daemia* (Forsk.) Chiov. (Asclepiadaceae) is used in *Ayurveda* for the treatment of inflammation, fever, strangury, asthma, diseases of *vata* and *kapha*. It is known as Uttaravaruni and Yugaphala in *Sanskrit.* The present study was undertaken to evaluate the analgesic activity of the aqueous and alcohol root extracts of *Pergularia daemia* (Forsk.) Chiov. using eddy's hot plate and heat conduction method. In eddy's hot plate method the aqueous extract showed significant analgesic activity at the doses of 500 mg/kg ($p<0.01$) and 1000 mg/kg ($p<0.001$) and alcohol extract showed significant analgesic activity at the doses of 500 and 1000 mg/kg ($p<0.001$). In heat conduction method both extracts showed significant analgesic activity at the doses of 500 & 1000 mg/kg ($p<0.001$) as compared to control group, when analyzed statistically by Tukey Kramer Multiple Comparison Test. The result obtained show that the aqueous and alcohol root extracts of *Pergularia daemia* (Forsk.) Chiov. possesses significant analgesic activity which confirms the traditional claims of the plant mentioned in Ayurveda.

Zulfiker et al[43] reported that fruits and whole herb of two medicinal plants, *Ficus racemosa* Linn. (Moraceae) and *Scoparia dulcis* L. (Scrophulariacae) were extracted in 95% ethanol to evaluate for centrally acting analgesic potential using hot plate and peripheral pharmacological actions using acetic acid induced writhing test in mice. The crude extracts of both the plants were found to have significant ($p<0.001$) analgesic activity at the oral dose of 100 & 200 mg/kg b. wt., in the tested models. In hot plate test *S. dulcis* showed increased latency period than *F. racemosa* whereas in acetic acid

induced writhing test *F. racemosa* showed reduced number of writhes than *S. dulcis* at two dose levels which are significant ($p<0.001$) compared to control. The results obtained support the use of fruits of *F. racemosa* and whole herb of *S. dulcis* in painful conditions acting both centrally and peripherally.

Ibrahim et al[44] reported that the work was aimed at investigating toxicity and analgesic potentials of the ethanol leaf extract of *Vernonia amygdalina* Del. in mice. Acute toxicity was evaluated by intraperitoneal administration of the extract whereas acetic acid-induced writhing in mice method was used for the analgesic study. The extract was found to have an LD50 of 288.5 mg/kg body weight *i.p.* and a significant ($p < 0.05$) dose-dependent analgesic activity with the highest percentage inhibition (71.9%) at 100 mg extract/kg against the acetic acid-induced writhing in mice. This activity was higher than that of the standard analgesic: *ketonal* (67.5%) used at 10 mg/kg. The results obtained showed that the plant has a relative toxicity to the mice and was found to have a dose-dependant analgesic property. The plant could therefore, serve as a potential source of analgesic.

3.5 Review of literature for Antipyretic:

Nagaveni et al[45] reported that the present study is designed to investigate preliminary photochemical characteristics and anti-pyretic effect of the Indian medicinal plant *Mangifera indica* which belongs to the family Anacardiaceae. The genus of this plant cited in Indian system of medicine, Ayurveda for the treatment of pain and fever associated various diseases. Naturally occurring terpenoids are unique phyto constituents of the genus *Mangifera indica*, but there is no scientific evidence has been demonstrated against the effect of this plant extract in pyretic *invivo* models. The ethanolic extract of mangifera indica bark shows potential antipyretic activity at a dose of 400 mg/kg.

Md. et al[46] reported that the main aim of this study is to evaluate antipyretic activity on *Murraya koenigii* spreng. leaves extracts. *Murraya koenigii* spreng. leaves were collected from rural area and extracted with Petroleum ether, Chloroform, Ethanol and the extract was screened for antipyretic activity by Yeast induced hyper pyrexia method. Paracetamol (150mg/kg) was used as standard drug. It was observed that Petroleum ether and chloroform extracts did not show significant decrease in elevated temperature with respect to corresponding control. The Ethanolic extract gives significantly reduced

elevated temperature. The present study indicates that *Murraya koenigii* spreng. leaves Ethanol extract possess good significant antipyretic activity as compared to control group, where as Petroleum ether extract, Chloroform extract.

Sushil et al[47] reported that the aqueous extract of *Swertia chirata* Buch Ham. Root (ASC) (Family: Gentianaceae) was evaluated for its antipyretic potential on Brewer's yeastinduced pyrexia in albino rats and Typhoid-Paratyphoid A, B vaccine induced Hyperexia in rabbits. In both models, the extract, at dose of 200 mg kg−1 body wt. and 400 mg kg−1 body weight, produced significant ($p<0.001$) reduction in elevated body temperature in a dose dependent manner. The antipyretic effect of the extract was comparable to that of paracetamol (150 mg kg−1 body weight, p.o.), a standard antipyretic agent.

Amiya et al[48] reported that the objective of the present work was to study the antipyretic activity of plant *Capparis zeylanica* Linn. belonging to family Capparaceae, known as "Karambha" in Sanskrit & "Asadhua" in Oriya. Materials & Methods- The Methanolic extract was taken for the study and evaluated for antipyretic activity using Brewer's yeast induced pyrexia in Wister strain albino rats. The methanolic extract at a dose of 100mg/kg & 200mg/kg were evaluated for antipyretic activity. Result - The extract of *C. zeylanica* plant showed a significant ($P < 0.01$) dose dependent antipyretic effect in yeast induced elevation of body temperature in experimental rats. Conclusion - The Methanolic extract of *Capparis. zeylanica* Linn. plant have significant antipyretic activity when compared with the standard drug. So. It can be recommended for further studies.

3.6 Review of literature for Anti-inflammatory activity:

Tailor et al[49] reported that in the present Study of Ethanolic extract of Stem of *Rubia cordifolia* Linn.(Rubiaceae) was screened for anti-inflammatory activity in carrageenan induced paw oedema rats. The effect was assessed by Difference in paw oedema volume, before & after the low & high dose administration of the extract in Rats. Ethanolic extract of *Rubia cordifolia* stem (20 & 40 mg./kg./ml.) were administered orally. Anti-inflammatory effects were compared with Standard drug- Indomethacin (10mg./kg/ml.). These observations helped us to conclude that Ethanolic Extract high dose is endowed with anti-inflammatory property.

Maminur et al[50] reported that previous phytochemical analysis of methanolic extract of *Acalypha indica* L. has indicated the presence of steroid, flavonoid and terpenoid types of compounds. Since these compounds are of pharmacological interest, coupled with the use of this plant in traditional medicine, prompted us to check *A. indica* L. for possible analgesic and antiinflammatory activities. The methanolic extract of *A. indica* L. showed statistically significant ($P<0.001$) analgesic activity in mice in a dose-dependent manner. A sustained and significant ($P<0.001$) inhibition of carrageenan-induced inflammation of rat paw was observed with 125 mg/kg and 250 mg/kg body weight. The methanolic extract of *A. indica* L. also demonstrated antiinflammatory effect in a dose-dependent manner. Maximum inhibition by the extract was observed at 250 mg/kg body weight after three hours of ingestion, which was comparable to that of the standard drug phenylbutazone at a dose of 100 mg/kg body weight. The obtained results provide a support for the use of this plant in traditional medicine.

Amar et al[51] reported that 2H-1,4-pyridooxazin-3(4H)-one was synthesized by condensation of 2-amino-3-hydroxy-pyridine with chloroacetylchloride by using standard procedure . Esterification with ethylchloroacetate and further condensation with hydrazine hydrate produced hydrazide of 2H-1,4-pyridooxazin-3(4H)-one. Schiff base derivatives of 2H-1,4- pyridoxazin-3(4H)-one were synthesized by the acid catalyzed condensation 2-(3-oxo-2,3-dihydro-4H-1,4- pyridoxazin-4-yl)acetohydrazide with various benzaldehyde derivatives. Schiff base derivatives were characterized by FT-IR, 1H-NMR. All compounds were screened for anti-inflammatory activity by carrageenan induced edema in mice paw against diclofenac sodium as a standard drug. The activity reflects their ability to provide protection (45.00-70.00%). The safety of these schiff base derivatives are reflected by toxicity studies.

Biswa et al[52] reported that the bark of *Xeromphis spinosa* extracted by a mixture of equal proportions of petroleum ether, ethyl acetate and methanol at an oral dose of 200 and 400 mg/ kg body weight exhibited significant anti-inflammatory activity when compared with control.

4. Material and Methods

4.1 Pharmacognostic Investigations

The systematic pharmacognostic investigation of the plant material and its documentation will help the scientific community for further investigation. So efforts are made to contribute in that direction.

A. Collection and Authentication of the whole plant.

B. Microscopic characters.

C. Proximate values.

a. Extractive values

- **Alcohol soluble extractive value.**
- **Water soluble extractive value.**

b. Moisture content determination

c. Ash values

- **Total ash**
- **Acid insoluble ash**
- **Water soluble ash**
- **Sulphated ash**

4.2 Phytochemical Investigations

The Fruit and Stem of *P. aculeata* and Leave of *R. aquatica* will subject to following phytochemical investigations:

A. Extraction

- **Extraction with Methanol.**
- **Cold maceration for Aqueous Extraction.**

B. Qualitative chemical identification tests.

C. HPTLC fingerprint profile

4.3 Pharmacological Screening

Pharmacological Screening

A) Animal selection:

The experiment will carry out using Swiss albino mice and rats of either sex weighing between 20-30gm for acute toxicity study and Swiss albino mice and rats of either sex weighing around 20-30 gms and 150- 180 g will use for the biological activity. Animals will maintained at normal laboratory conditions and will give standard animal feed.

B) Acute Toxicity Studies[53]:

Albino mice of either sex weighing between 20-30gm will use during investigation. The animals will fast overnight. The OECD guideline no-420 fixed dose method will adopt and accordingly doses of total alcoholic and aqueous extract of Fruit and Stem of *P. aculeata* and Leave of *R. aquatica* will calculate.

As per following the OECD guideline no. 420 fixed dose method procedure, the safest dose of total alcoholic extract and aqueous extract will 2000mg/kg body weight. The safe dose will found to be 2000mg/kg body weight; hence $1/10^{th}$ of the dose will take as effective dose.

C) Extract used:

Total methanol extract, aqueous extract of Fruit and Stem of *P. aculeata* and Leave of *R. aquatica* will screen for their biological property. The dried alcoholic extract and aqueous extract will suspend in distilled water using tween 80.

4.1.1 Analgesic Activity[54-55]:

A very large number of methods will introduce for the laboratory assessment of analgesic activities. This is partly a reflection of the difficulties inherent in using animal preparations in the search for capable of alleviating pain in man. These difficulties can be summarized as follows:

(a) It is impossible to know whether the sensation experienced by animal is response to noxious stimuli is qualitatively similar to the pain felt by human being in similar circumstances.

(b) The relief of pain in human beings may involve more than an interference with pain producing mechanisms or an inhibition of activity in the nervous pathway for pain i.e. physiological factors play prominent part.

(c) The most serious deficiency concerns the nature of the stimulus used to provoke pain.

1. Peripheral analgesic activity

Most of the so called peripheral analgesic possess anti-inflammatory properties and in some cases also antipyretic activity besides analgesia. For many of them the mode of action will elucidate as an inhibition of cyclooxygenase in the prostaglandin pathway. Nevertheless, new peripheral analgesics will test not only for their in vitro activity on cyclooxygenase but also for their in vivo activity.

2. Central analgesic activities.

Pain is a symptom of many diseases requiring treatment with analgesics, that means opioids drugs. The addiction liability of opioids led to intensive research for compounds without this side effect. Many approaches will use to differentiate the various action of strong analgesic by developing animal models not only for analgesic activity but also for addiction liability. Several types of opioids receptors will identify in the brain allowing in vitro binding tests. However the in vitro tests can only partially substitute for animal experiments involving pain. Pain is a common phenomenon in animals at least in vertebral animal similar to that felt by man.

Analgesic effects in animal are comparable with the therapeutic effect in the man. Needless to say, that in every instance painful stimuli to animal must be restricted as much as possible. Painful stimuli can be consist of direct stimulation of the efferent sensory nerves or stimulation of pain receptor by various means such as heat or pressure. The role of endogenous peptides such as encephalin and endorphins gives more insight into brain processes and the action of central analgesics.

Pain can also be elicited by inflammations. Progress has been made in elucidating the role of various endogenous substances such prostaglandins and peptides in the inflammatory process. Most of the so called non-steroids anti-inflammatory agents have also analgesic activity. An old but excellent survey on methods being used to test compounds for analgesic activity has been provided. Today the classification into central and peripheral analgesic is definitively too simplified but provides a guide for differentiation by pharmacological methods.

(a) Tail Immersion Method:

Principle:

The method will develop to be selective for morphine-like compounds. The procedure is based on the observation that morphine-like drugs are selectively capable of prolonging the reaction time of the typical tail-withdrawal reflex in rats induced by immersing the end of the tail in warm water of 55 ^{0}C

Procedure:

Young female Wister rat (150-180g body weight) will place into individual restraining cages leaving the tail hanging out freely. The animals will allow to adapt to the cages for 30 min before testing the lower 5cm portion of the tail is mark. This part of the tail is immersed in a cup of freshly filled water of exactly 55° C within a few seconds the rat reacts by withdrawing the tail. The reaction time is record in interval of 0.5s by a stopwatch. After each determination the tail will carefully dried. The reaction time will determined before and periodically after either oral or subcutaneous administration of test substance e.g. after 0.5, 1, 2, 3, 4 and 6h. The withdrawal time of untreated animals will between 1 and 5.5s. A withdrawal time of more than 6sec therefore was regarded as a positive response.

(b) Hot Plate Method:

Principle:

The paw of the mice and rats was very sensitive to heat at temperatures which was not damaging the skin. The response was jumping, withdrawal of the paws licking of the paws. The time until these responses occur was prolonged after administration of

centrally acting analgesics, whereas peripheral analgesics of the acetylsalicylic acid or phenyl-acetic acid type do not generally affect these responses.

Procedure:

Groups of 6 mice of either sex with an initial weight of 20 to 30 gm were used for each dose. The hot plate, which is commercially available, consists of an electrically heated surface. The temperature is controlled for 55^0c to 56^0c. This can be copper plate and a heated glass surface. The animal will place on the hot plate and time until either licking or jumping occurs is recorded oral or subcutaneous administration of the standard or the test compound.

4.1.2. Anti-pyretic activity[56]:

Materials and Methods:

Preparation of extract

Total methanol extract, aqueous extract, *P. aculeata* and *R. aquatica* will screen for its biological property. The dried alcoholic extracts and aqueous extracts will suspend in distill water using teen will employ to assess biological activity.

Animals used

Albino rats of either sex weighing 150-180 gm each will use for this experiment. The animals will house in standard metal cages and provided with food and water add libitium.

Preparation of standard:

Paracetamol was used as standard drug (positive control). Paracetamol (150 mg/kg body wt) was dissolved in normal saline.

Induction of yeast-induced pyrexia

Rats were divided into eight groups of six each for this experiment. The normal body temperature of each rat will measure rectally at predetermined intervals and record. Fever will induced per the method described by Smith and Hamburger (1935). The rats will train to remain quiet in a restraint cage. A thermistor probe will insert 3-4 cm deep into the rectum and fastened to the tail by adhesive tape. The temperature will measure on a

thermometer. After measuring the basal rectal temperature, animals will give a subcutaneous injection of 10 ml/kg body wt. of 15% w/v yeast suspend in 0.5% w/v methyl cellulose solution. Rats will then returned to their housing cages. After 19 h of yeast injection, the animals will again restrained in individual cages for another recording of their rectal temperature as described above.

Drug administration

After 19 h of yeast injection, the extracts will administer orally at doses of 200 mg/kg body wt. to six groups of animals, respectively. A similar volume (5ml/kg body wt.) of normal saline solution was administered orally to the control group. The eighth group of animals received the standard drug Paracetamol (150mg/kg body wt.) orally. Rats will restrain for recording of their rectal temperature at the nineteenth hour, immediately before Extracts, saline or Paracetamol administration, and again at one-hour intervals up to the twenty-third hour after yeast injection.

Statistical analysis

All the results were expressed as mean ±SEM. ANOVA by Dunnett Compare all Vs Control.

4.1.3. Anti-Inflammatory activity[49-52,57]:

Carrageenan Induced Rat Paw Oedema- (Acute Model):

Preparation of extract

Total methanol extract, aqueous extract, *P. aculeata* and *R. aquatica* will screen for its biological property. The dried alcoholic extract and aqueous extract will suspended in distilled water using Tween 80 as an emulsifying agent and was employed to assess biological activity.

Animals use.

Albino rats of either sex weighing 180-200 g each will use for this experiment. The animals will house in standard metal cages and provided with food and water *ad* libitium.

Preparation of standard:

Acute inflammation will induce by injecting carrageenan (0.1 ml of 1% suspension in 0.9% saline) in sub-plantar region and paw volume will measured at 0, 1, 2 and 4 hours

Assessment of Anti- inflammatory activity

After grouping separately, each group will orally administered with one of the following treatments.

All the treatments will administer 30 min prior to carrageenan, except sodium iodide which was give 20 minutes prior to carrageenan. Acute inflammation will induce in each group by injecting 0.1 ml of 1% carrageenan into the sub plantar region of right hind paw. A mark will put on the leg at the mallaleous to facilitate the dipping of the leg to the same level at the second and subsequent times.

The initial reading will take as zero hour, that is, immediately after injecting carrageenan and the procedure was report at zero an hour, one, two, three and four hours after carrageenan injection. The difference between zero hour reading and one of the subsequent readings provides the actual Edema volume at that time. The mean paw volume at different times was calculate for all groups and the percentage inhibition will then calculated by using the formula;

$$\% \text{ Inhibition} = 1 - \frac{V_t}{V_c} \times 100$$

Where V_t = the amount of oedema formed in drug treated group.

V_c = the amount of oedema formed in the control group.

The results were analyzed by ANOVA followed by Dunnet's test (p-value ≤ 0.05 was taken as significant)

5. Patent and research publications:

Patent will be proceeding as per confirmation of Isolated compound from our selected medicinal plants.

5.1 Research Publication will be published in following below reputed journals:

Sr. No.	Name of The Journals
1.	Inventi rapid: Planta activa
2.	Asian journal of Biochemical and Pharmaceutical Research
3.	Journal of Pharmaceutical Science and Bio scientific Research
4.	Ancient Science of Life
5.	International Journal of Indigenous medicinal plants
6.	International Journal of Research in Pharmacy in Pharmacy and science
7.	International Journal of Pharmaceutical Innovation

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